Invited commentary for JCI

Chronic diarrhea, bile acids and *Clostridia*

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Abbreviations: BA, bile acid; BAD, bile acid diarrhoea; C4, 7α-OH-4-cholesten-3-one; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FD, functional diarrhea; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; GCDCA, glycochenodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; IBS-D, irritable bowel syndrome with predominant diarrhea; PBAD, primary bile acid diarrhoea; SeHCAT, ^{75}Se-homocholic acid taurine; UDCA, ursodeoxycholic acid; 7-KDCA, 7-ketodeoxycholic acid

Abstract (150 word limit)

Excessive fecal bile acid (BA) loss causes symptoms in a large proportion of people diagnosed with irritable bowel syndrome with diarrhea, a common functional bowel disorder. This bile acid diarrhea (BAD) results from increased hepatic synthesis of BAs, with impaired negative feedback regulation by the ileal hormone fibroblast growth factor 19 (FGF19). In this issue of the *JCI*, Zhao et al. investigated BA metabolism, including fecal BAs, serum BAs and FGF19, in patients and controls. They identified associations with fecal bacterial BA metabolism and specific microbiota, especially *Clostridium scindens.* These findings have been tested in a mouse model using microbiota transplants and antibiotic treatment. This group of organisms has potential as a biomarker for BAD and to be a target for therapy.

Pathophysiology of the functional bowel disorders

Bile acids (BAs) are increasingly recognized as major factors in the pathophysiology of the functional bowel disorders with diarrhea (1,2). Recent guidelines recommend investigation of patients with chronic diarrhea to detect the presence of BA diarrhea (BAD) (3,4). Such patients may meet the diagnostic criteria for irritable bowel syndrome with predominant diarrhea (IBS-D) or functional diarrhea (FD), depending on how they report abdominal pain (1), but they all experience frequent, soft or liquid stools, with urgency and often fecal incontinence, with a response to BA sequestrants (5). Diagnostic delay is common, as the recognition of BAD by physicians is poor, and there is limited availability of diagnostic tests, such as the nuclear medicine ^{75}Selenohomocholic acid taurine (SeHCAT) test (4,6). SeHCAT is unavailable in the U.S.A., which has led to the development of alternative blood or fecal tests (7). The frequency of BAD is estimated at 28% in IBS-D or FD, giving a population prevalence of 1% (8-10).

The precise causes of this idiopathic, primary BAD (PBAD) have largely been unclear. BA are mostly reabsorbed in the ileum and undergo an enterohepatic circulation. BA malabsorption, which is clearly present in ileal Crohn's disease or resection, is usually not found in PBAD. An alternative cause for the excess fecal BA loss is increased hepatic BA synthesis (6,11). BAs are synthesized from cholesterol by the enzyme, cholesterol 7α-hydroxylase, (CYP7A1). Raised levels of the BA intermediate 7α-OH-4-cholesten-3-one (C4) demonstrate the increased activity of the classical synthesis pathway, where CYP7A1 is rate-limiting. This enzyme is regulated via negative feedback by the ileal hormone fibroblast growth factor 19 (FGF19). Serum FGF19 levels are usually low in PBAD, and are inversely correlated with C4 (12). In ileal tissue, BA and other agonists bind to the receptor known as the farnesoid X receptor (FXR), to stimulate FGF19 transcription (13). In PBAD, there is evidence for impaired FGF19 production from the ileum (14). Further, PBAD is associated with certain polymorphisms in genes (*FGFR4* and *KLB*) that affect the hepatic response to FGF19 (15).

A possible cause of primary bile acid diarrhea

In this issue of the *JCI,* Zhao et al. investigated the relevance of the BA-FXR signalling pathway and the effects of microbial metabolism of BA in relation to the possible cause of PBAD (16). Starting with an unselected population of 345 Chinese adults who met Rome IV criteria for IBS-D (1), the authors identified around 25% of these with excess total fecal BA (called the BA^{+}IBS-D group by Zhao et al.). This figure is a similar proportion to the IBS-D patients reported elsewhere with PBAD (8-10). Importantly, the authors showed that the BA-positive group, when compared to the BA-negative group, also showed raised serum C4 and total BA levels, with lower FGF19. Total fecal BAs were associated with increased symptom severity, increased BA synthesis and impaired FGF19 feedback, making the Zhao et al. study patients representative of those studied previously (16).

BA profile differences in serum and fecal samples of the BA-positive group suggest that there are differences in the BA-transforming microbiota in PBAD. In the serum, BAs conjugated with glycine or taurine and the free unconjugated liver-derived (primary) BAs, cholic acid (CA), and chenodeoxycholic acid (CDCA) predominated. CDCA and glycochenodeoxycholic acid (GCDCA) were raised, and the secondary BA, ursodeoxycholic acid (UDCA) and its conjugated form,

glycoursodeoxycholic acid (GUDCA) were also particularly higher in the BA-positive group. Similarly, in feces, increases in the total of most BAs, and the proportions of CA, CDCA, UDCA and 7-ketodeoxycholic acid (7-KDCA) were found. Relevantly, identifying raised levels of unconjugated primary BA (CA and CDCA) in fecal samples has been proposed as a novel diagnostic method to identify PBAD (7).

Intestinal bacteria possess specialized BA modifying enzymes, and consequently are integral to BA metabolism. Deconjugation, removing glycine or taurine, by choloylglycine hydrolase genes (*cgh*, also known as bile salt hydrolases), and 7-dehydroxylation/dehydrogenation by *bais* genes (7-dehydroxylases) and *hdhA* (7α-hydroxysteroid dehydrogenase, 7-HSDH) are key to the production of the secondary, modified BAs. Using shotgun metagenomics and DNA extracted from the feces of the study group, Zhao et al. found reduced *Bacteroidetes*, with decreased abundances of *Alistipes* and *Bacteroides*, which express *cgh*, contributing to the reduced activity, and less bile acid deconjugation in the BA-positive patients (the BA^{+}IBS-D group). This group had an increase in their microbiota in *Firmicutes*, including *Clostridia* and especially *Clostridium scindens*, with significantly higher abundance of 7-dehydroxylation/dehydrogenation genes. The abundance of *C. scindens* was positively associated with total fecal BA and serum C4, and, notably, was inversely related to serum FGF19 levels (16).

A key step for the investigators involved recapitulating these findings in mice. Human fecal microbiota was transplanted into pseudo germ-free mice, and after one week, those that had received feces from the group of BA-positive patients had cecal microbiota with decreased deconjugating ability, increased 7-HSDH and 7α–dehydroxylating levels. They also had pathophysiologic differences, increased fecal water and BA, increased serum C4 and hepatic BA-synthesis enzyme activity and decreased ileal FGF15 (the mouse homolog of human FGF19). No differences in ileal BA transporters or hepatic receptors was identified. The *Firmicutes*/*Bacteroidetes* ratio was higher, and increases in *Clostridium cluster XIVa* and *C. scindens* were found (16).

Next, modifications to the microbiota of conventional mice were made. The researchers either directly administered *C. scindens* into the stomachs or, alternatively, treated the mice with vancomycin, an antibiotic that inhibits *Clostridium* species. Levels of 7-HSDH and 7α–dehydroxylating activity changed in keeping with the previous findings, as did BA and gene expression. *C. scindens* enhanced BA synthesis and excretion, reducing ileal FGF15 at both transcript and protein levels. Conversely, vancomycin increased ileal *Fgf15* and inhibited hepatic *Cyp7a1* gene expression (16).

Cell-line studies were performed to add to these findings. In the hepatocyte L-02 cell-line, vancomycin or *C. scindens* failed to produce direct effects on CYP7A1 expression. In NCI-H716 cells, which have an enterocyte-like phenotype, CDCA stimulated FGF19 expression, as previously demonstrated in similar cells (17), confirming findings with human ileal tissue that FXR agonists regulate this gene potently (13). Further, coincubating the cells with other BAs, including GUDCA, GCDCA, GCA, UDCA or 7-KDCA, inhibited the CDCA stimulated FGF19 (16)..

Other research has suggested that certain modified, secondary BA can have actions as FXR antagonists. For example, tauro-β-muricholic acid was shown to act as an antagonist (18), but humans, unlike mice, have very low levels of this BA. In the Zhao et al. study, the related metabolite, ω-muricholic acid, was detected at higher levels in feces of BA-positive patients. GUDCA also has FXR

antagonist effects, and is increased by metformin, which decreases a specific *Bacteroides* and can also cause BAD (19,20).

Clinical implications

The findings in Zhao et al. are significant and important, advancing our understanding of the role of the gut microbiota and BAs in IBS-D. They will need to be confirmed in other defined groups of PBAD, such as those diagnosed by reduced SeHCAT retention, blood tests or by raised fecal total or primary BA (6,7). They add to previous PBAD mechanistic studies, where overproduction of BAs with raised C4, due to impaired FGF19-related negative feedback, are central (6). Reduced deconjugation of BAs gives higher levels of fecal primary BAs (7), but increased 7-dehydroxylation/dehydrogenation produces secondary BAs, which can be reabsorbed and then conjugated. If these conjugated secondary BAs, like GUDCA, inhibit FXR, their increase could explain the reduced ileal FGF19 production.

The microbial and enzymatic changes associated with IBS-D and outlined by Zhao et al. need considering alongside other possible factors (Figure)(16). For example, lower basal and stimulated FGF19 transcript levels have been shown in patient ileal mucosa explant cultures, implying an inherent defect can predispose individuals to PBAD (14). There is also evidence that Diet1 protein regulates FGF19 protein secretion, and that *Diet1* genetic variants associate with PBAD (21). Further, variants in FGF19 hepatic response genes (*FGFR4* and *KLB*) may also contribute to PBAD (15).

We now have a target group of organisms, which might be considered to be a biomarker for BAD. This may allow the development of a quick molecular test to detect levels of *C. scindens* relative to other bacteria in fecal samples. Moreover, since the human population's gut microbiota sits along a continuum with different ratios of *Firmicutes*/*Bacteroidetes*, it also raises the possibility that we could be able to predict which individuals, enriched with *Firmicutes*, who could have a higher prevalence of PBAD and/or IBS-D with high levels of BA. Is this population of *Firmicutes*, and specifically the enhanced *Clostridia* and *C. scindens*, maintained by the higher BA levels or due to other unknown factors? There is still much work to be done to prove the relevance of these findings and the exact relationships of these microbiota with BA metabolism and PBAD in individuals with IBS-D. Nevertheless, antibiotic treatment trials, with vancomycin or others targeting *Clostridia* rather than *Bacteroidetes*, may be beneficial in PBAD and should be one of the many outcomes of this notable study.

Figure legend

Factors contributing to BA diarrhea

The key physiological steps in BA metabolism (black) produce or interact with specific factors (blue) that can contribute to the pathophysiology of altered BA metabolism and thus result in chronic diarrhea. Bacterial species influence various BA proportions (varying arrow width) and influence FGF19 signaling (blue arrow) See text for abbreviations.

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